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U-Shaped Association between Plasma Manganese Levels and Type 2 Diabetes

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ABSTRACT

Background: Manganese is both an essential element and a known toxicant, and plays important roles in many mechanisms in relation to type 2 diabetes (T2D). But epidemiological studies are rare.

Objective: To investigate the association of plasma manganese with newly diagnosed T2D as well as whether the association could be modified by MnSOD polymorphisms.

Methods: We conducted a case-control study of 3228 participants in China: 1614 T2D patients and 1614 controls. Concentrations of plasma magnesium were measured and all participants were genotyped for MnSOD Val16Ala polymorphism (rs4880).

Results: A U-shaped association was observed between plasma manganese and T2D, with increased ORs in relation to either low or high plasma manganese levels. Compared with middle tertile, the multivariate-adjusted ORs (95%CI) of T2D associated with lowest tertile and highest tertile of plasma manganese were 1.89 (1.53-2.33) and 1.56 (1.23-1.97), respectively. In spline analysis, the U-shaped association was consistently indicated, with the lowest odds of T2D at the plasma manganese concentration of 4.95 μg/L. Minor allele frequencies (C allele) of MnSOD Val16Ala polymorphism (rs4880) in NGT and T2D groups were 13.57% and 14.50%, respectively. The MnSOD rs4880 polymorphism was not associated with T2D and no interaction was found between plasma manganese and MnSOD rs4880 polymorphism in relation to T2D.

Conclusions: Our results suggested a U-shaped association between plasma manganese and T2D; both low and high levels of plasma manganese were associated with higher odds of newly diagnosed T2D. The U-shaped association was not modified by MnSOD rs4880 polymorphism.

Introduction

Manganese is an essential micronutrient required for normal carbohydrate, lipid and protein metabolism (Aschner and Aschner 2005). Manganese is involved in normal immune functions, bone growth, regulation of blood glucose and cellular energy, and it is a key component of manganese superoxide dismutase (MnSOD) (Aschner and Aschner 2005). As a major antioxidant due to its mitochondria matrix localization, MnSOD plays a critical role in protecting mitochondria and islets from elevated reactive oxygen species (ROS) (Chen et al. 2005; Fridovich 1995), which may serve as an important trigger of insulin resistance and type 2 diabetes (Anderson et al. 2009; Hoehn et al. 2009; Houstis et al. 2006). Despite its essentiality, at excessive levels manganese is toxic to humans, especially the central nervous system (CNS) (Guilarte 2010; Sidoryk-Wegrzynowicz and Aschner 2013), which plays an important role in glucose homeostasis and type 2 diabetes (T2D) (Schwartz et al. 2013).

In animal models, several studies have elucidated that insufficient levels of dietary manganese could result in suboptimal levels of MnSOD activity (Burlet and Jain 2013; Lee et al. 2013), lower insulin secretion (Baly et al. 1985), and glucose uptake and metabolism (Baly et al. 1990). Consistently, manganese supplementation could enhance MnSOD activity and protect against T2D and diabetes complications (Burlet and Jain 2013; Lee et al. 2013). However, with limited samples, several epidemiologic studies have yielded inconsistent associations between manganese levels and T2D (Kazi et al. 2008; Koh et al. 2014; Rambouskova et al. 2013). As a

transition metal, manganese itself is an oxidant at high levels and appears to be involved in oxidative damage and mitochondrial dysfunction, which has been implicated in the development of T2D (Lowell and Shulman 2005; Maechler and Wollheim 2001). Although excessive manganese has been reported to be associated with neurodevelopment and neurological disorders (Claus Henn et al. 2010; Guilarte 2010; Sidoryk-Wegrzynowicz and Aschner 2013), epidemiologic evidence regarding the association between excessive manganese and T2D has not been reported.

MnSOD gene as well as levels of manganese could affect the activity of MnSOD (Bresciani et al. 2013). The MnSOD Val16Ala polymorphism (rs4880), in exon 2 of the human MnSOD gene located on chromosome 6q25, is considered as the most interesting polymorphism in the MnSOD gene, because the T allele instead of the C allele could result in less MnSOD activity and less efficient transport of MnSOD into the mitochondrial matrix (Shimoda-Matsubayashi et al. 1996; Sutton et al. 2003). The MnSOD Val16Ala polymorphism has been shown to be associated with diabetes and diabetic complications, but the findings are inconsistent (Katakami et al. 2014; Mollsten et al. 2007; Mollsten et al. 2009; Tian et al. 2011).

To our knowledge, no study has been conducted to examine the association of both low and high levels of manganese with T2D in humans, and it is unclear whether the association differs according to MnSOD genetic variations. We therefore sought to investigate the association of

plasma manganese with newly diagnosed T2D as well as whether the association is modified by the MnSOD Val16Ala polymorphism in a large case-control study.

METHODS

Study population

The study population consisted of 3228 participants: 1614 newly diagnosed T2D patients and 1614 normal glucose tolerance (NGT) individuals. The patients of newly diagnosed T2D were consecutively recruited from those attending for the first time the outpatient clinics of Department of Endocrinology, Tongji Medical College Hospital, Wuhan, China, from January 2009 to December 2011. Concomitantly, we recruited healthy NGT individuals who were frequency-matched by age (±5 years) and sex to patients from an unselected population undergoing a routine health check-up in the same hospital. The inclusion criteria of NGT and newly diagnosed T2D were: age ≥ 30 years, body mass index (BMI) < 40 kg/m^2, no history of a diagnosis of diabetes and no history of receiving pharmacological treatment for hyperlipidaemia or hypertension. Patients with clinically significant neurological, endocrinological or other systemic diseases, as well as acute illness and chronic inflammatory or infective diseases, were excluded from the study. All the participants enrolled were of Chinese Han ethnicity. They gave informed written consent to the study and did not take any medication known to affect glucose tolerance or insulin secretion before participation. The study was approved by the ethics committee of the Tongji Medical College.

Assessment of NGT and T2D

The definitions of T2D met the respective diagnostic criteria recommended by the World Health Organization in 1999 (Alberti and Zimmet 1998). T2D was diagnosed when FPG ≥ 7.0 mmol/l and/or 2-h post-glucose load ≥ 11.1 mmol/l. A FPG concentration < 6.1 mmol/l, and a 2-h oral glucose tolerance test (OGTT) plasma glucose concentration < 7.8 mmol/l was considered NGT.

Body composition and blood parameters

Demographic and health information was collected by using a questionnaire, including age, gender, current smoking status, current alcohol consumption, physical activity level (hours per week), history of disease (hypertension and hyperlipidemia) and family history of diabetes. Height (m) and weight were measured using standardized techniques. BMI was calculated as weight divided by the square of height (kg/m^2). After a 10-h overnight fast, all participants underwent a 75 g OGTT, and venous blood samples were collected at 0 and 2-h for determination of FPG, OGTT2h, fasting plasma insulin (FPI), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C). Homoeostasis model assessment insulin resistance (HOMA-IR) score was computed using the following formula: FPI [m-units (milliunits)/L]×FPG (mmol/L)/22.5. The index of HOMA of β-cell function (HOMA-B) was calculated as (20×FPI)/(FPG-3.5) (Matthews et al. 1985).

Measurement of plasma manganese concentrations

Plasma manganese concentrations were measured in MOE Key Lab of Environment and Health at School of Public Health at Tongji Medical College of Huazhong University of Science and Technology using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700 Series, Japan). The samples of T2D and NGT groups were randomly assayed in everyday measurement. For quality assurance, the CRM (certified reference material) ClinChek No. 8883 and No. 8884 human plasma controls were used. For No. 8883, we determined a concentration of 6.52 ± 0.28 μg/L (certified: 6.72 ± 1.34 μg/L), and for No. 8884, we measured 17.5 ± 0.51 μg/L (certified: 16.9 ± 3.4 μg/L). The intra-assay and inter-assay coefficient of variation of plasma manganese were both < 5%. All participants had plasma manganese levels above the detection limit (0.001μg/L).

Genotyping

The MnSOD polymorphism rs4880 was genotyped using an allelic discrimination assay-by-design TaqMan method on an ABI 7900HT PCR system (Applied Biosystems, Foster City, CA). The primers and labeled oligonucleotide probes were designed and supplied by Applied Biosystems. The TaqMan genotyping reaction was amplified (50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min), and the end point fluorescent readings were performed by ABI 7900HT data collection and analysis software version 2.2.1

(SDS 2.2.1). The genotype success rate was 98.12% for rs4880, and Hardy–Weinberg equilibrium tests were performed.

Statistical analysis

General demographic and laboratory characteristics are summarized as mean ± SD or median with interquartile range (IQR), depending on the normality of the continuous variables, or as number with proportion for categorical variables. To test for differences of characteristics among different glucose regulation status, continuous variables were compared using one-way ANOVA, and a Chi-square test was used for categorical variables. For calculation of the odds ratio (OR) for T2D, plasma manganese concentrations were treated as continuous variables and categorized in tertiles according to the NGT group: tertile 1, ≤ 4.21 μg/L, tertile 2, 4.21-6.84 μg/L, and tertile 3, ≥ 6.84 μg/L. Binary logistic regression analysis was used to assess the associations of T2D with plasma manganese concentrations. ORs and 95% confidence intervals (CIs) were adjusted for known risk factors for T2D, including age, sex, BMI, current smoking status, current alcohol consumption, physical activity levels (never or rare, 1 to 2, 3 to 4, ≥5 hours/week), hypertension, family history of diabetes, plasma iron, plasma copper, and plasma selenium . Stratified analyses were conducted by age, sex, BMI, current smoking status, current alcohol consumption, physical activity levels, hypertension and family history of diabetes. To further explore the potential nonlinearity of the relationship between plasma manganese concentration and T2D, a logarithmic transformation was used to improve the normality of plasma manganese distributions, and we

used restricted cubic splines with 4 knots at the 20th, 40th, 60th, and 80th percentiles of log-plasma manganese concentration, excluding values outside the 5th and 95th percentiles via Stata version 12 (Stata Corp). The distributions of rs4880 genotypes were analyzed for deviation from Hardy-Weinberg equilibrium using a likelihood ratio test. Binary logistic regression analysis was also used to assess the associations of T2D with rs4880 polymorphisms, in log-additive and dominant models. In addition, we examined the association between plasma manganese concentration (tertiles) and T2D stratified by rs4880 polymorphisms (CC, CT, CC+CT vs. TT genotypes), as well as the association between rs4880 polymorphisms and T2D according to plasma manganese tertiles.

To test the interaction between plasma manganese concentrations and rs4880 polymorphisms in association with T2D, we introduced a multiplicative interaction term of genotypes (CC, CT, CC+CT vs. TT genotypes) and plasma manganese tertiles as continuous variables and added these variables to the same aforementioned multivariate model. Likelihood ratio test with one degree of freedom was used to assess the significance of the interaction with a comparison of the likelihood scores of the two models with and without the interaction term. All data analyses were performed with SAS 9.1 (SAS Institute Inc. Cary, NC, USA). P values presented are two-tailed with a significant level at 0.05.

RESULTS

General anthropometric and metabolic characteristics of the 3228 participants (1614 T2D and 1614 NGT) are summarized in Table 1. Compared to control subjects, the individuals with T2D had higher BMI, greater prevalence of family history of diabetes and hypertension, higher levels of TC, TG, FPG, FPI, and OGTT2h. Lower HOMA-β and higher HOMA-IR were observed in the T2D group compared to the controls. Medians (IQR) of plasma manganese concentration were 5.26 μg/L (3.67-8.33) for NGT and 4.37 μg/L (2.73-7.62) for T2D. Minor allele frequencies (C allele) of rs4880 in NGT and T2D groups were 13.57% and 14.50%, respectively. The genotype distributions of rs4880 were in Hardy–Weinberg equilibrium for both T2D ($P = 0.39$) and NGT ($P = 0.80$) groups.

Table 2 presents logistic regression results for T2D associated with the levels of plasma manganese concentrations categorized into tertiles according to the distribution in controls. Compared to the middle tertile, the multivariate-adjusted ORs (95%CI) of T2D associated with the lowest tertile and the highest tertile of plasma manganese were 1.89 (1.53-2.33) and 1.56 (1.23-1.97), respectively. In the spline regression analysis, the nonlinear spline terms were statistically significant ($P < 0.01$ for nonlinearity); a U-shaped association was observed between plasma manganese and T2D, with the lowest odds of T2D at the plasma manganese concentration of 4.95 μg/L (Figure 1). In stratified analysis according to age, sex, BMI, current smoking status, current alcohol consumption, physical activity, family history of diabetes and

hypertension, the U-shaped association was consistently observed in all subgroups (Table 3), and significant interactions were found between plasma manganese concentration and age ($P < 0.01$) as well as physical activity ($P < 0.01$). The Mn-diabetes associations for low and high levels of plasma manganese were stronger in age ≥ 55 group compared with age < 55 group. The association for low level of plasma manganese was stronger than high level in group without physical activity, while an opposite manner was observed in group with physical activity.

There was no significant association between rs4880 and T2D in either main or stratified analysis by age, sex, BMI, physical activity and plasma manganese levels (see Supplemental Material, Table S1 and Table S2). The U-shaped Mn-diabetes association was observed in every rs4880 genotype group, and no interaction was found between plasma manganese and rs4880 in relation to T2D (P for interaction = 0.54, see Supplemental Material, Table S3).

DISCUSSION

To our knowledge, this was the first population-based study showing that the association between plasma manganese and T2D followed a U-shaped manner; both low and high levels of plasma manganese were associated with higher odds of newly diagnosed T2D. In addition, the U-shaped association was not modified by the MnSOD Val16Ala polymorphism.

The association between manganese and T2D is likely to be complex because manganese is both an essential nutrient and a potential toxicant, depending on the amount of exposure. Likewise, our results suggested that both low and high levels of manganese were associated with

increased risk of T2D, which was consistent in all stratified subgroups. Prior studies have reported conflicting results for the relationships between manganese and T2D. A recent study in Korean reported that the prevalence of self-reported diabetes significantly increased in participants with the lowest quartile blood manganese (Koh et al. 2014). However, in that study, high blood manganese levels were found to be consistently associated with high blood pressure, but not associated with diabetes. Two previous case-control studies also indicated that diabetic patients had lower blood levels of manganese than controls in other populations (Forte et al. 2013; Kazi et al. 2008; Koh et al. 2014), while diabetic individuals showed no elevation of manganese levels than controls in other studies (Ekmekcioglu et al. 2001; Rambouskova et al. 2013). In contrast, Anetor et al found that serum manganese level was double higher in diabetic patients compared with non-diabetics (Anetor et al. 2007). Additionally, significant interactions were found between plasma manganese concentration and age as well as physical activity in this study, which were not reported before and remained to be validated in other studies.

The inconsistent findings between this study and previous studies might be related to the large sample size and wide range of manganese levels in this study, which allowed us to examine the association of both low and high levels of manganese with T2D. In our study, medians (IQR) of plasma manganese concentration were 5.26 μg/L (3.67-8.33) for NGT and 4.37 μg/L (2.73-7.62) for T2D, higher than reference values (0.79 ± 0.63 μg/L) of adults reported by the Agency for Toxic Substances Registry (ATSDR. 2012) actually based on only one study with a small sample (n = 68) (Rukgauer et al. 1997). Several following studies evaluated plasma manganese

levels in the healthy and diabetic persons, but the results varied seriously between studies. A study in Nigeria found that plasma manganese level was significantly elevated in diabetic patients (209 ± 0.39 μg/L) compared with non-diabetics (99 ± 0.28 μg/L) (Anetor et al. 2007), but another study in Austria showed a nonsignificant elevation of plasma manganese in diabetic individuals than controls (1.81 ± 1.38 μg/L vs. 1.57 ± 0.98 μg/L) (Ekmekcioglu et al. 2001). Currently there is no internationally acceptable value or range for plasma manganese concentration in the general population. The discrepancies between populations remain to be elucidated, because plasma manganese concentrations could be affected by its exposure levels, effects of genetic predisposition and other predisposing factors on its metabolism, between-laboratory differences in methods (ICP-MS vs. electrothermal atomic absorption spectrometry) and measurement errors, or variations in population characteristics among studies. Physical activity was a widely acceptable lifestyle to prevent T2D (Aune et al. 2015; Jeon et al. 2007), whilst it was an important factor on manganese metabolism by generating large numbers of ROS (Watson 2014), which might explain the interaction between plasma manganese concentration and physical activity in this study. Meanwhile, the effect on manganese metabolism of age and chronic manganese toxicity might explain the interaction of age on Mn-T2D association (Aschner and Aschner 2005; Burton and Guilarte 2009). Due to limited sample and study design, no previous study was undertaken to investigate the interactions. Further studies with large samples, especially prospective studies, are warranted to confirm the association between manganese levels and T2D.

The U-shaped association between plasma manganese and T2D is biologically plausible. Firstly, levels of manganese could affect metallation and activity of MnSOD (Lee et al. 2013; Paynter 1980). Suboptimal MnSOD related to insufficient levels of manganese could result in increased mitochondrial ROS formation, which may directly cause macromolecular damage or indirectly result in oxidative stress by activating stress-sensitive pathways such as NFκB, p38 MAPK, JNK/SPAK, and hexosamine (Evans et al. 2003). Activation of these pathways has been shown to lead to significant deterioration of glucose-stimulated insulin secretion (GSIS), mitochondrial dysfunction and β-cell dysfunction (Anderson et al. 2009; El Khattabi and Sharma 2013; Hirosumi et al. 2002; Kamata et al. 2005). Accordingly, manganese supplementation may enhance MnSOD activity and protect against diabetes by enhancing insulin secretion (Burlet and Jain 2013; Lee et al. 2013). On the other hand, excessive manganese could lead to increased level of MnSOD, selectively decreasing superoxide ion ($O_2^{\bullet -}$) levels at the expense of increased hydrogen peroxide (H_2O_2) production. Transient exposure of β-cells to 200 μM H_2O_2 may decrease the secretory response to glucose accompanied by β-cell dysfunction (Li et al. 2009), and long term exposure to high levels of H_2O_2 may also lead to insulin resistance (Anderson et al. 2009; Bashan et al. 2009). Peroxisome-generated H_2O_2 has been shown to be involved in sensing signals that disrupt β-cell function (Elsner et al. 2011), and mitochondrial H_2O_2 emission was proposed as a regulator linking excess fat intake to insulin resistance in the skeletal muscle of both rodents and humans (Anderson et al. 2009).

Secondly, independent of MnSOD, manganese supplementation was found to down-regulate ROS in both vitro and vivo studies (Burlet and Jain 2013), which suggested there might be another mechanism for manganese related to diabetes. However, manganese itself is an oxidant at high level. Neurotoxicity from manganese overexposure appears to involve oxidative damage to dopaminergic neurons in particular, as well as mitochondrial dysfunction (Burton and Guilarte 2009; Desole et al. 1994; Dobson et al. 2003). It was indicated that excessive manganese could compete with magnesium binding sites in proteins (Mukhopadhyay and Linstedt 2011), which also enhances oxidative stress. Thirdly, manganese is an essential mineral nutrient for glucose metabolism and other functions by activating certain enzymes or through other manganese-dependent metalloenzymes (Aschner and Aschner 2005). On the other hand, excess manganese could induce adverse effects, especially on the central nervous system (Burton and Guilarte 2009; Sidoryk-Wegrzynowicz and Aschner 2013), which is also implicated in glucose homeostasis and diabetes (Schwartz et al. 2013). Finally, it was postulated that manganese accelerates cellular glucose uptake by potentiating insulin action and that manganese may act on the pancreas by stimulating the release of stored insulin into the bloodstream or by inhibiting the release of glucagon (Rubenstein et al. 1962). However, more studies are warranted to clarify the mechanisms underlying the association between manganese and diabetes.

As MnSOD is the major mitochondrial antioxidant and plays a critical role in protecting mitochondria and islets from ROS, many studies have elucidated the role of MnSOD and its encoding gene in relation to T2D and diabetes complications (Bresciani et al. 2013). The

MnSOD Val16Ala polymorphism is the most commonly studied polymorphism, because the T allele instead of the C allele could translate the valine amino acid (GTT) into alanine (GCT). This translation leads to 30-40% less of MnSOD activity and less efficient transport of MnSOD into the mitochondrial matrix (Sutton et al. 2003). However, the sample sizes of previous epidemiologic studies were rather small, and the results have been inconsistent (Bresciani et al. 2013; Flekac et al. 2008; Lee and Choi 2006; Liu et al. 2009; Nakanishi et al. 2008). A meta-analysis showed a significant protective effect of the C allele on risk of T2D (Tian et al. 2011), but this association disappeared after excluding one study that deviated from Hardy–Weinberg equilibrium (HWE) (Flekac et al. 2008). In the current study, the MnSOD Val16Ala polymorphism was not associated with T2D. To our knowledge, this is the largest population study to comprehensively investigate the association between the Val16Ala polymorphism and T2D. Moreover, we found that the MnSOD genotypes did not modify the association between plasma levels of manganese and T2D risk. ROS are indicated to be an important trigger of T2D, but T2D is as well hypothesized to be accelerated or even caused by shortages in cellular ROS (Watson 2014). Although it is acknowledged that elevated MnSOD could protect against diabetes by down-regulating ROS in β-cells and enhancing insulin secretion, there is still some debate as to whether increased MnSOD expression has beneficial or deleterious effects on muscle insulin sensitivity (Anderson et al. 2009; Hoehn et al. 2009). The frequency of the Ala (C) allele was rather low (~14%) in this study, so there remains a need to validate the association

between the MnSOD Val16Ala polymorphism and T2D, as well as the interaction with manganese in large prospective studies.

The strengths of our study included the large number of participants and objectively measured plasma manganese levels. Our subjects with T2D were confined to the newly diagnosed and drug naïve because anti-diabetic or drugs may alter the status of manganese metabolism. Moreover, we defined diabetes based mainly on fasting and postprandial glucose levels from an OGTT.

Our study also has several limitations. First, the case-control nature of our study does not allow us to infer any causality between plasma manganese and T2D because plasma manganese level may be affected by the development of insulin resistance and T2D. Second, our measurement of manganese was confined to plasma compartment. We used plasma manganese concentration as a biomarker to measure manganese status to avoid potential bias through dietary assessment, such as systematic measurement error in self-reported dietary exposure and the influence of other nutrients on the bioavailability of manganese (Davis et al. 1992; Finley 1999). Thirdly, although we controlled for multiple T2D risk factors including several oxidative stress related minerals (copper, selenium, iron) in plasma, we could not rule out the possibility that other correlated nutrients also contributed to the observed association. We also lacked of information on education level, plasma zinc concentration and inflammatory markers that might also confound our results. In addition, all participants in this study were of Chinese Han ethnicity, which minimizes the confounding effects by ethnic background, but may limit the

generalizability of the results to other ethnic groups. Furthermore, we did not measure the concentrations of manganese according to different valence states, which may have different effects on ROS formation, lipid peroxidation and ensuing cell death (HaMai et al. 2001).

CONCLUSIONS

Our study demonstrated a U-shaped association between plasma manganese concentrations and T2D in a Chinese population, and the association was not modified by the MnSOD Val16Ala polymorphism. Further studies are warranted to confirm our findings in prospective cohorts and elucidate the potential mechanisms underlying the relationship between manganese and T2D.

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Table 1 Anthropometric and metabolic characteristics of NGT and T2D groups.

Parameters	NGT	T2D	*P*
N	1614	1614	
Age (y)	54.69±10.41	52.54±9.79	0.06
Male, n (%)	910(56.38)	922(57.12)	0.63
BMI (kg/m^2)	23.56±3.44	24.96±3.61	<0.01
Current smoker, n (%)	490(30.36)	477(29.55)	0.03
Current drinker, n (%)	450(27.88)	462(28.62)	0.01
Family history of diabetes, n (%)	114(7.06)	311(19.27)	<0.01
Hypertension, n (%)	394(24.41)	565(35.01)	<0.01
Fasting plasma glucose (mmol/L)	5.27±054	9.44±3.13	<0.01
OGTT2h (mmol/L)	6.43±0.92	17.08±5.03	<0.01
Hemoglobin A1c	5.58±0.44	8.60±3.43	<0.01
Triglycerides (mmol/L)	1.16(0.84-1.65)	1.44(1.05-2.08)	<0.01
Total cholesterol (mmol/L)	4.39(3.57-5.14)	4.61(3.98-5.32)	<0.01
Fasting plasma insulin (μU/mL)	7.62(4.67-11.75)	8.88(5.83-13.36)	<0.01
HOMA-β	80.12(50.36-120.39)	35.32(17.69-61.04)	<0.01
HOMA-IR	1.79(1.11-2.79)	3.63(2.32-5.33)	<0.01
Manganese (μg/L)	5.26(3.67-8.33)	4.37(2.73-7.62)	<0.01
MnSOD SNP rs4880			0.38
Allele C	438(13.57)	468(14.50)	
Allele T	2790(86.43)	1184(85.50)	
CC genotype	30(1.86)	38(2.35)	0.50
CT genotype	378(23.42)	392(24.29)	
TT genotype	1206(74.72)	1184(73.36)	

Abbreviations: BMI, body mass index; HOMA-beta, homeostasis model assessment of beta cell function; HOMA-IR, homeostasis model assessment of insulin resistance; IGR, impaired glucose regulation; NGT, normal glucose tolerance; OGTT2h, 2-h post-glucose load; T2D, type 2 diabetes mellitus; MnSOD, manganese-dependent mitochondrial superoxide dismutase.

Data were presented as number (percentage) for categorical data, mean (standard deviation) for parametrically distributed data or median (interquartile range) for nonparametrically distributed data.

Table 2 Association of plasma manganese concentrations with T2D.

Variables	Tertiles of plasma manganese concentrations (μg/L)		
	1 (Lowest)	2	3 (Highest)
	≤4.21 μg/L	4.21-6.84	≥6.84 μg/L
N(NGT/T2D)	538/780	541/377	535/457
Crude	2.08 (1.75-2.47）	1	1.23 (1.02-1.47）
Model 1	2.08 (1.73-2.49）	1	1.28 (1.05-1.55）
Model 2	2.15 (1.75-2.63）	1	1.54 (1.23-1.92）
Model 3	1.89 (1.53-2.33）	1	1.56 (1.23-1.97）

Model 1, adjusted for age, sex and BMI. Model 2, adjusted for Model 1, current smoking status, current alcohol drinking status, physical activity, family history of diabetes and hypertension. Model 3, adjusted for Model 2, plasma iron, plasma copper, and plasma selenium.

Table 3 Adjusted ORs for plasma manganese levels associated with T2D in subgroups.

Groups	Tertiles of plasma manganese concentration (μg/L)			P value for interaction
	1 (Lowest)	2	3 (Highest)	
Sex				
Male	2.14(1.62-2.83)	1	1.28(0.96-1.72)	0.06
Female	2.31(1.69-3.17)	1	2.06(1.47-2.90)	
Age				
< 55	1.81(1.34-2.45)	1	1.30(0.94-1.79)	<0.01
≥ 55	2.45(1.80-3.33)	1	1.77(1.28-2.45)	
BMI				
< 24	2.17(1.62-2.90)	1	1.47(1.07-2.01)	0.45
≥ 24	2.26(1.70-3.02)	1	1.67(1.24-2.26)	
Physical activity				
No or rare	1.97(1.52-2.56）	1	1.37(1.02-1.82）	<0.01
Yes	1.55(1.06-2.25）	1	2.11(1.38-3.22）	
Current smoking				
Yes	2.21(1.50-3.26)	1	1.18(0.79-1.75)	0.21
No	2.22(1.74-2.84)	1	1.76(1.36-2.29)	
Current drinking				
Yes	1.84(1.22-2.78)	1	1.27(0.84-1.93)	0.49
No	2.36(1.85-3.00)	1	1.73(1.34-2.24)	
Family history of diabetes				
Yes	1.95(1.09-3.49)	1	1.31(0.71-2.43)	0.73
No	2.29(1.84-2.86)	1	1.62(1.29-2.05)	
Hypertension				
Yes	2.62(1.81-3.80)	1	1.42(0.97-2.10)	0.12
No	2.07(1.61-2.66)	1	1.66(1.27-2.15)	

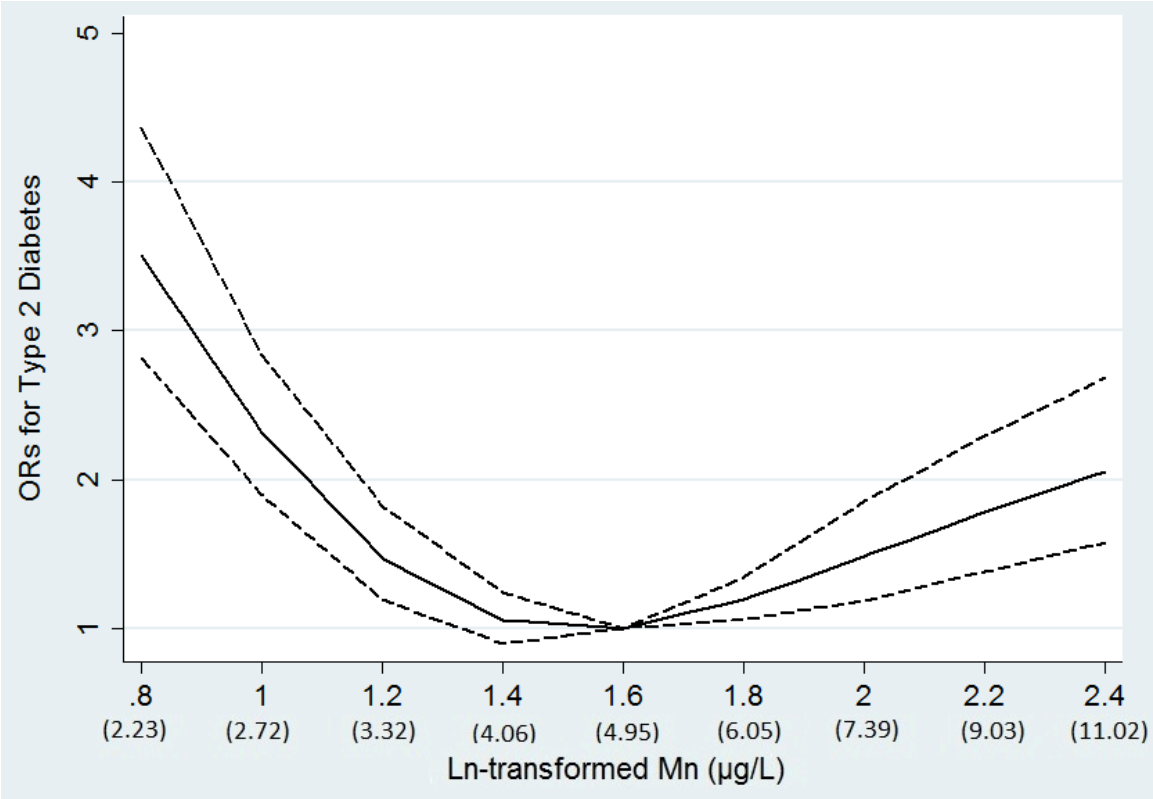
Adjusted for age, sex, BMI, current smoking status, current alcohol drinking status, physical activity, family history of diabetes, hypertension, plasma iron, plasma copper, and plasma selenium.

FIGURE LEGENDS

Figure 1 Adjusted ORs (solid line) and 95% CIs (dashed line) for T2D by ln-transformed plasma manganese concentrations.

Results were adjusted for age, sex, BMI, current smoking status, current alcohol drinking status, physical activity, family history of diabetes, hypertension, plasma iron, plasma copper, and plasma selenium.

Figure 1.


ORs for Type 2 Diabetes
1
2
3
4
5
.8
(2.23)
1
(2.72)
1.2
(3.32)
1.4
(4.06)
1.6
(4.95)
1.8
(6.05)
2
(7.39)
2.2
(9.03)
2.4
(11.02)
Ln-transformed Mn (µg/L)